EXPRESSION OF BRAF MUTATION IN THYROID NEOPLASMS.

DISSERTATION

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CERTIFICATE

CERTIFICATE

This is to certify that the dissertation work entitled "**EXPRESSION OF BRAF MUTATION IN THYROID NEOPLASMS"** submitted by Dr. H.Volga is work done by her during the period of study in the department of Pathology, PSGIMS & R from June 2009 to April 2012. This work was done under the guidance of Dr. S.Shanthakumari, Professor, Department of Pathology.

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One can count the seeds in a fruit. But none can count the fruits in a seed.

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INTRODUCTION

INTRODUCTION

The thyroid gland is situated anterior to trachea in lower part of the neck. It consists of right and left lobe connected across the median plane by isthmus. The thyroid parenchyma is composed of lobules of thyroid follicles with intervening thin fibrous tissue septae.Each follicle consists of central core of colloid surrounded by single layer of follicular epithelial cells. The parafollicular 'C' cells are located at the periphery of the follicles in small groups or as single cells. [1]

The main function of thyroid gland is production of thyroid hormones T3 and T4 which regulates the cellular metabolism, oxygen consumption and protein synthesis. The parafollicular cells secrete calcitonin which in turn regulates the calcium level in the plasma by a feedback mechanism [1].

Thyroid neoplasms account for 1% of all malignancies and it is the most common among all endocrine malignancies [2].Environmental and genetic factors play a major role in thyroid neoplasms due to the dependence on iodine for hormone production. This organ is also vulnerable to the genotoxic effects of radioactive iodine and to the non-genotoxic effects caused by iodine deficiency [3], thereby resulting in neoplastic lesions.

With improved, technically advanced diagnostic screening procedures like guided FNA procedures, PET scan etc, detection of early neoplastic lesions are on the rise .When early neoplastic lesions are sampled from thyroid, morphological assessment to diagnose these, pose a significant challenge to pathologist and for treatment by the surgeons.

Many a time morphology and immunohistochemistry is adequate for diagnosing neoplasms of thyroid, [4] but a significant number of thyroid neoplasms go unnoticed when early lesions are sampled. As genetic alteration is the earliest change that occurs in oncogenesis and it may be acquired in the due course of life, it is important to identify these for early treatment, prognostication and for prevention. Therefore the knowledge of genetic alteration will help not only in detecting the genesis of thyroid carcinomas but also might help in treatment and prognostication.

Recent data's suggest that alterations in RAS-RAF-MAP kinase signaling pathway is seen in many neoplastic lesions of various organs but is more frequent in thyroid carcinomas. In papillary thyroid carcinoma BRAF mutation especially V600E mutation plays a vital role .Studies state [5, 6] that BRAF mutation is specific for papillary thyroid carcinoma and is frequently seen in high grade tumors and is associated with poorer outcomes.

We did a Pubmed search for articles on relevant areas. The number of articles returned for contain keywords in Pubmed search is Nil. (Limits; 10 years, English language, humans).keywords used in MESH “Proto-oncogene proteins B-raf/genetics” “Thyroid Neoplasms” and “India”.

Thus there is paucity of Indian literature in the area of pathogenesis of neoplasms of thyroid. The fact that there is a need to establish and conduct scientific work linking proteins , genes and finding molecular markers for thyroid neoplasms emphasizes the importance of our work. As 1% of population world over suffers from thyroid neoplasms, research in this area would help in treatment, prognostication and possibly prevention of this major problem.

Therefore we propose to study the prevalence of BRAF V600E mutation in thyroid neoplasms in PSGIMS&R and its association with various phenotypic features as an initial step.

AIMS AND OBJECTIVES

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The aims of this study are

1. To estimate the prevalence of BRAF V600E mutation in thyroid neoplasms diagnosed at a tertiary care hospital at Coimbatore.
2. To correlate the BRAF V600E mutation with clinicopathological parameters.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The thyroid gland first appears as a median anlage and two lateral anlagen. The median anlage develops in the floor of primitive pharynx at the foramen cecum and grows caudally to become bilobed forming the greater portion of the thyroid gland and forms follicular epithelial cells. The two lateral anlagen derived from ultimobranchial bodies' fuse with median thyroid anlage and become incorporated into the lateral lobes .Then the ultimobranchial bodies undergo dissolution phase and forms peripheral component of cell groups called C cells [1].

Thyroid neoplasms are the most common endocrine neoplasms and accounts for 1% of all human malignancies [2]. It usually occurs in the young and in the middle age group. It is more frequent in females [2, 3]. The relative frequency of papillary carcinoma is high in regions of adequate or high dietary iodine intake. In regions of iodine deficiency the incidence of papillary carcinoma is high after iodine supplementation [3]. In endemic goiter regions the prognosis for thyroid carcinoma is worse, when compared with regions with an adequate dietary iodine intake [7]. Even in regions with endemic goiters papillary neoplasms predominate over follicular cancers [3].

A report from Health and family Welfare Department, Government of Tamilnadu [8] from their survey conducted from 1990, states that goiter is prevalent in all the districts. 18 districts out of 29 have more than 10 % prevalence. Coimbatore is included among the 18 districts, where the goiter prevalence is 11.7%.

Studies from India state that papillary thyroid carcinoma is more common in coastal areas. A report by Professor, N.Dorairajan, on investigating thyroid cancer [9] states and I quote "In India, thyroid cancer has a widespread distribution with certain subtypes, notably papillary cancer, occurring in coastal areas of Tamil Nadu, Andhra Pradesh and Kerala which are iodine rich. The iodine content of soil modifies development of these cancers."He also states "In South India, excess iodine in diet is related to the higher incidence of papillary cancer compared to other more malignant subtypes of thyroid cancer."

The thyroid neoplasms are classified as given below.

CLASSIFICATION OF THYROID TUMORS (WHO 2004) [3]

BENIGN

- Follicular adenoma

MALIGNANT

- Papillary carcinoma
- Follicular carcinoma
- Medullary carcinoma
- Poorly differentiated carcinoma (Anaplastic)
- Undifferentiated carcinoma (Insular)
- Mucinous carcinoma
- Muco- epidermoid carcinoma
- Squamous cell carcinoma
- Others

FOLLICULAR ADENOMA:

Follicular adenoma is a benign tumor enveloped by a thick fibrous capsule. Morphologically, it is composed of closely packed follicles, trabeculae or solid sheets of cuboidal cells with pale or darkly stained nuclei and inconspicuous nucleoli.

Rare variants like atypical follicular adenoma, hyalinizing trabecular adenoma and Signet- ring cell follicular adenoma [10] are also reported. Increase in cellularity, mitoses, spontaneous necrosis or infarction but lack of invasion into the capsule or vasculature are the characteristic features of atypical follicular adenoma.

Hyalinizing trabecular adenoma is characterized by elongated tumor cells arranged in a wavy trabecular pattern around capillaries. Nuclear grooves, pseudoinclusions and peri nuclear haloes are prominent. Predominance of signet ring cells with abundant cytoplasmic vacuoles, intermixed with groups of follicular cells of normal cytologic features are the findings reported in Signet- ring cell follicular adenoma.

Hurthle cell adenomas are considered a subtype of follicular adenoma. They are bright brown in a gross appearance. This neoplasm is characterized by the presence of Oxyphilic/Hurthle cells arranged in follicular and or trabecular pattern with partial or complete encapsulation morphologically; the Hurthle cells are large cells with abundant eosinophilic granular cytoplasm and a round nucleus. Accumulation of abundant mitochondria gives a granular appearance to the cytoplasm under the light microscope. It has a tendency for spontaneous infarction [11].

PAPILLARY CARCINOMA:

The most common of all primary thyroid malignancies is papillary carcinoma, accounts for about 70- 85% of cases [12]. It can occur in any age and has a female preponderance. Multifocal disease along with intrathyroidal extension and metastasizes to regional lymph nodes are the characteristic features. Extra thyroidal extension can occur and extend

beyond the capsule of the thyroid gland to involve structures like larynx, trachea or esophagus.

Papillary carcinoma classic type is characterized by formation of complex arborizing papillae with a central fibro vascular core under the light microscope. The papillae are covered by cells with crowded oval nuclei. The nuclei show margination of chromatin, overlapping, with nuclear grooving and intra nuclear cytoplasmic pseudo inclusions. However, papillary thyroid carcinoma can exhibit a pure follicular pattern or mixed papillary and follicular pattern. Psammoma bodies are nothing but the lamellated concretions formed by deposition of calcium is one of the most important finding in papillary carcinoma [13].

The relationship between Hashimoto's thyroiditis and papillary carcinoma is controversial. Reports by Livolsi [14] suggested that the lymphocytic infiltration of the surrounding thyroid tissue is induced through autoimmune mechanisms triggered during the development of papillary carcinoma. Harach et al [15] found that lymphocytic thyroiditis more commonly associated with papillary thyroid carcinoma than with other types of thyroid carcinomas. But a study conducted by Dubravka [16] et al showed no significant association between Hashimoto's thyroiditis and Papillary carcinoma.

VARIANTS OF PAPILLARY CARCINOMA:

Several morphological variants of papillary carcinoma have been recognized based on the architecture, growth pattern, cellular morphology and stromal features. The subtypes are important as some of these subtypes are more aggressive in their biologic and clinical behaviour irrespective of their bland appearance. [11]

I. Papillary microcarcinoma:

Papillary micro carcinomas usually measures 1 cm or less in diameter and often unencapsulated. It is detected incidentally in thyroidectomy specimens for other indications and is associated with an excellent prognosis though occasional regional lymph node metastasis is usual [11].

II. Follicular variant:

This neoplasm composed of small to medium sized, irregularly shaped follicles with abortive papillary formation. Stromal sclerosis and psammoma bodies can be present. Characteristic nuclear features of papillary carcinoma are evident. Distant metastasis and vascular invasion are common. Some of these tumors exhibit encapsulation with an exceptionally good prognosis (so called Lindsay tumor) [11].

III. Tall cell variant:

This is a rare variant and is composed of tumor cells whose heights are at least three times their widths. Tall cells must be seen in 50% or more of the tumor areas to make a diagnosis of tall cell variant of papillary thyroid carcinoma [11] Necrosis, mitotic activity and extra thyroidal extension are common. These tumors occur in older patients, often males, with a more aggressive clinical behaviour. This variant is commonly associated with BRAF mutation reflects its aggressiveness [17].

IV. Oncocytic variant:

This variant of papillary carcinoma comprising of complex branching papillae with thin fibrovascular stromal core, covered by polygonal oncocytic cells with abundant granular eosinophilic cytoplasm.[13]The behaviour is similar to conventional papillary carcinoma.

V. Warthin tumor like variant:

The characteristic feature of this variant is brisk lymphoplasmacytic infiltrate in the papillary stalks. Papillae were lined by oncocytic cells with the typical nuclear features of classic papillary carcinoma. It is frequently associated with Hashimoto thyroiditis [11]. The immuno profile of the lymphoid cells is no different from chronic lymphocytic thyroiditis.

VI. Cribriform variant:

Predominant cribriform pattern, focal papillary architecture, solid and spindle cell areas interspersed with squamoid morules, characterize this uncommon variant of papillary carcinoma [12]. The nuclei frequently harbor eosinophilic, homogenous cytoplasmic inclusions. It typically occurs in patients with familial adenomatous polyposis or Gardner syndrome. This tumor is often multifocal and occurs in young women.

VII. Diffuse sclerosing variant:

This rare variant of papillary carcinoma seen more frequently in children and is associated with a poor prognosis [12]. The typical finding of this tumor includes diffuse involvement of both the lobes, dense lymphoplasmacytic infiltrate with extensive lymphatic permeation.

VIII. Clear cell variant:

Tumor cells have extensive clear or vacuolated cytoplasm. Cytoplasmic clearing is usually due to accumulation of glycogen [10]. The nuclear features are otherwise typical of papillary carcinoma.

FOLLICULAR CARCINOMA:

It accounts for 5-15% of all thyroid malignancies. The tumor can be encapsulated or widely invasive. A true capsular and / or a vascular invasion

in a follicular neoplasm are mandatory to diagnose follicular carcinoma. It is more common in an older age group and more common in females. [10, 13]

The criteria to diagnose follicular carcinoma include invasion of the capsule, invasion through the capsule and invasion into vein in or beyond the capsule [11].

Tumors with limited focal capsular and / or vascular invasion that are apparent only on histological examination are termed as 'Minimally invasive follicular carcinomas'. 'Follicular tumors of uncertain malignant potential' are designated, if the presence or absence of invasion is not certain or unequivocal.

Tumors with infiltrative margins and extensive vascular invasion (>4 blood vessel) are the features of widely invasive follicular carcinoma [10, 11].

Follicular carcinomas lack multifocality and do not invade lymphatics. But it can metastasize hematogenously to bone and lungs. The metastatic lesions from follicular carcinoma are histologically similar to the primary neoplasm in the thyroid. It can also be deceptively bland and mimic normal thyroid tissue.

MEDULLARY CARCINOMA:

Medullary carcinoma is a malignant tumor of C cell origin and is of great diagnostic importance because of its aggressiveness. It comprises less than 10% of all malignant thyroid malignancies [11]. Calcitonin secretion is characteristic. It occurs in the setting of several inherited cancer syndromes including multiple endocrine neoplasia (MEN) syndromes. Medullary carcinoma can exhibit trabecular, insular or sheet like growth patterns traversed by delicate fibro vascular septa. The cells are small with stippled chromatin. The tumor stroma characteristically contains Amyloid. Tumor necrosis and mitotic figures are infrequent. Lymphatic invasion and extra thyroidal involvement by direct extension can be present.

POORLY DIFFERENTIATED CARCINOMA:

Poorly differentiated thyroid carcinoma or insular carcinoma usually occurs in an older age group and it represents a heterogeneous group of malignant neoplasms, with varied growth patterns and biological behaviour. It grows in the form of nests and solid to microfollicular arrangement. The cells are small, uniform with hyper chromatic or vesicular nucleus and variable mitotic activity [13].Prominent vascularization, coagulative necrosis, infiltrative growth pattern and obvious vascular invasion are characteristic.

Age more than 45 years; tumor necrosis and mitotic count of more than 3 per 10 high power fields have been associated with the aggressive biological behavior.

ANAPLASTIC CARCINOMA:

Anaplastic carcinoma or undifferentiated thyroid carcinoma is a rare aggressive tumor accounts for about 5 - 10% of all malignant tumors of thyroid [10].The tumor is usually seen in older patients and in iodine deficient areas with a rapidly enlarging mass with compression symptoms. Anaplastic carcinoma exhibits a wide range of morphologic patterns and cell types, predominant being epithelioid cells, spindle cells and giant cells with focal squamoid differentiation. The tumor cells exhibit marked anaplasia and also shows frequent mitoses, extensive coagulative necrosis and marked degree of invasion into the surrounding soft tissues [3]. Distant metastasis is frequent. A pre-existing well differentiated thyroid neoplasm, more often follicular or papillary carcinoma is usually seen in many, if not in most of the undifferentiated carcinomas.

TNM CLASSIFICATION AND STAGING OF THYROID CARCINOMAS

The staging of the disease explains the spread of cancer and grading gives the level of differentiation, in combination, these determine clinical gravity of the disease. The TNM system is endorsed by the International Union against Cancer (UICC) and the American Joint Commission on Cancer (AJCC) are commonly used. The staging of thyroid neoplasms is as given below [3]

T- Primary Tumor

TX: Primary cannot be assessed.

T0: No evidence of primary tumor.

T1: Tumor 2 cm or less in greatest dimension, limited to thyroid.

T2: Tumor more than 2 cm but not more than 4 cm in greatest dimension, limited to thyroid.

T3: Tumor more than 4 cm in greatest dimension, limited to thyroid or any tumor with minimal extra thyroidal extension (e.g. extension to sternothyroid muscle or perithyroid soft tissues) limited to the thyroid.

T4a: Tumor extends beyond the thyroid capsule and invades any of the following: subcutaneous soft tissues, larynx, trachea, esophagus, recurrent laryngeal nerve*

T4b: Tumor invades prevertebral fascia, mediastinal vessels, or encases carotid artery*

T4a* (Anaplastic carcinoma only) Tumor (any size), limited to the thyroid**

T4b* (Anaplastic carcinoma only) Tumor (any size), extends beyond the thyroid capsule***.

Notes:

Multifocal tumors of all histological types should be designated (m).

* All Anaplastic/undifferentiated thyroid carcinomas are considered T4.

** Intrathyroidal anaplastic carcinoma – considered surgically resectable.

*** Extra thyroidal anaplastic carcinoma – considered surgically unresectable.

N-Regional Lymph Nodes

NX: Regional lymph nodes cannot be assessed.

N0: No regional lymph node metastasis.

N1: Regional lymph node metastasis.

N1a: Metastasis in Level VI (pretracheal and paratracheal, including prelaryngeal and Delphian lymph nodes).

N1b: Metastasis in other unilateral, bilateral or contra lateral cervical or Upper/ superior mediastinal lymph nodes.

M – Distant Metastasis

MX: Distant metastasis cannot be assessed.

M0: No distant metastasis

M1: Distant metastasis

STAGE GROUPING:

For papillary and follicular, medullary and anaplastic/ undifferentiated carcinomas separate stage groupings are recommended. Unlike most other cancers, thyroid carcinomas are grouped into stages in a way that considers both the subtype of the neoplasm and the patient's age [3].

Papillary or Follicular under 45 years

Stage I	Any T	Any N	M0
Stage II	Any T	Any N	M1

Papillary or Follicular, 45 years and older and Medullary of any age

Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1, T2, T3	N1a	M0
Stage IVA	T1, T2, T3	N1b	M0
	T4a	N0, N1	M0
Stage IVB	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

Anaplastic/ Undifferentiated (all are considered stage IV)

Stage IVA	T4a	Any N	M0
Stage IVB	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

GRADING:

Tumor grading in thyroid malignancies is of little significance as more than 95% of cases are well differentiated using standard grading criteria [3]. Certain variants such as tall cell variant and diffuse sclerosing variants of papillary thyroid carcinoma are associated with an aggressive clinical behaviour. Biological behaviour of follicular carcinoma can be assigned based on tumor size, local extension and presence of distant metastasis. Poor survival of medullary carcinoma [13] is determined by the presence of necrosis, squamous metaplasia and distant metastasis.

MOLECULAR ALTERATIONS IN THYROID MALIGNANCY:

Hundreds of cancer associated genes have been discovered over the past two decades. The identification of genes and pathways involved will not only enhance our understanding of the biology of this process, it will also provide new targets for early diagnosis and facilitate treatment design.

Cancers arise owing to the accumulation of mutations in critical genes that alter normal programmes of cell proliferation, differentiation and death.

Several molecules that are involved in the pathogenesis of thyroid cancers are emerging as diagnostic and or prognostic tool for patient management. Among all thyroid malignancies, papillary carcinomas

commonly have one of the following genetic alterations: BRAF point mutations RET/PTC rearrangements or RAS point mutations [18].

Follicular carcinoma is frequently associated with PAX8/PPARγ fusion gene and loss of heterozigosity on 3p and 7q loci as well as RAS mutations [18]. Activating germline point mutations of RET [17] is a feature of medullary carcinoma are present in 95% of patients with MEN 2.

Poorly differentiated and undifferentiated thyroid cancers have been associated with inactivating mutations of p53, CTNNB1 mutations, and BRAF and RAS mutations.RET rearrangement is associated with poorly differentiated thyroid tumor not in anaplastic carcinoma [19].

BRAF: AN OVERVIEW

BRAF is a Serine - Threonine kinase that belongs to the family of RAF (20) proteins. It acts upstream of the MEK1/2 kinases in response to RAS signals.

Structurally RAF protein is divided into two functional domains , the N - terminal regulatory domain and C- terminal regulatory domain with three conserved region (CR 1,2 and 3).CR1 and CR2 being present in N terminal domain and CR3 is situated in C terminal domain [21].

Normally in a cell RAS proteins are attached to the plasma membrane on the cytoplasmic aspect, the endoplasmic reticulum and the Golgi

membrane. They are activated by the growth factor binding to its receptors .In the inactive state, RAS proteins are bound to GDP. If there is any stimulation ,exchange of GDP to GTP occurs leading to conformational change that produces active RAS[2].The activated RAS binds to the RAS binding domain in CR1 of RAF and recruits RAF to the membrane .This activates downstream signaling cascade[21]. RAF phosphorylates the mitogen –activated protein kinase (MAPK).

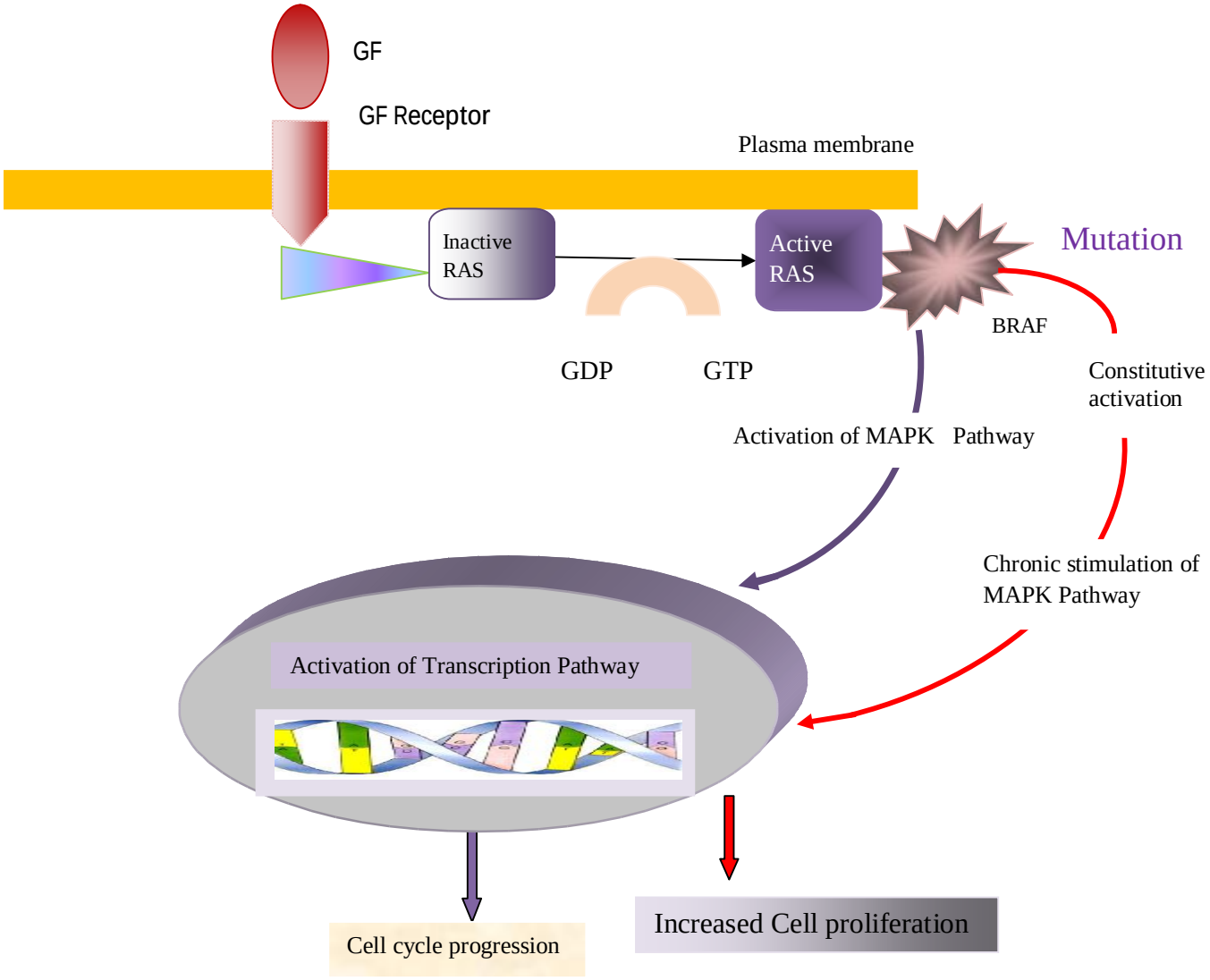
Extracellular signal-regulated kinases MEK 1 and 2 gets activated which in turn phosphorylates and activates extracellular signal regulated kinases ERK 1, and 2. Activated ERK migrates to the nucleus. In the nucleus, it activates various transcription factors leading to cell cycle progression which results in cell proliferation and differentiation [22] (Fig 1).

The RAF protein has three isoforms, A-RAF, B-RAF and C-RAF .Among these BRAF is commonly found in thyroid follicular cells [19]. B-RAF has a higher basal kinase activity when compared to other isoforms. Since serine 445 is constitutively phosphorylated in BRAF, a single mutation at codon 600 results in constitutive activation of BRAF in human cancers [21, 23].

BRAF protein is expressed in higher levels in hematopoietic cells, neurons, testicles and is the predominant isoform [19] in thyroid follicular

cells and is the most potent activator of the MAPK pathway. Gain-of-function BRAF mutation provides an alternative route for the aberrant activation of ERK signaling resulting in constitutive activation of BRAF kinase that means it is able to phosphorylate MEK as monomers in a RAS independent manner and chronic stimulation of MAPK pathway resulting in increased proliferation , decreased survival and differentiation of cells[24] (Fig 1).

Fig 1: **Mechanism of BRAF**



GF-Growth Factor; GDP-Guanosine diphosphate; GTP- Guanosine-triphosphate; MAPK-Mitogen-Activated Protein Kinase

This mechanism is implicated in the tumorigenesis of several human cancer for example malignant melanoma , thyroid carcinoma ,colorectal

carcinoma , ovarian carcinoma and carcinomas of biliary tract, ovary, colon , endometrium ,liver , breast, pancreas and cervix[21,25] .

BRAF EXPRESSION IN THYROID CARCINOMAS:

Many a time diagnosis of thyroid malignancy can be reached by morphological assessment alone. Immunohistochemical study will be useful if the tumors exhibit unusual patterns to confirm diagnosis and to establish the prognosis.

There has been a significant improvement in the knowledge of molecular alterations over the last two decades in all tumors including thyroid malignancies. Oncogenic BRAF activation represents the most prevalent molecular alterations.

BRAF mutations are involved in early thyroid carcinogenesis but it is not a germline mutation instead it is a somatic genetic alteration [26].

The BRAF mutation occurs early and plays an important role in the pathogenesis of papillary thyroid carcinoma in which point mutations of the BRAF gene are the most common mutation to occur in about 40 - 45 % [24]. According to some literature [27] incidence of BRAF mutation in papillary thyroid carcinoma is 35%-69% of PTC.

Most common mutation of BRAF is V600E. Here the missense thymine(T) to adenine (A) transversion at nucleotide 1799 in exon 15 occur, resulting in the substitution of a valine by glutamate at residue 600 . The less common mutation being the K601E mutation found in thyroid cancer[28].

Many studies have found that BRAF mutation is associated with poorer clininopathologic outcome [17, 29, 30]. A study conducted by Henderson et al found that recurrent papillary thyroid carcinoma is significantly associated with predominant BRAF mutation [31].

Among various subtypes BRAF mutation occurred most commonly in tall cell variant of PTC followed by conventional PTC and less commonly in follicular variant of papillary thyroid carcinoma. The Tall cell variant being the aggressive tumor indicating that BRAF mutation associated with poorer outcome [26, 32].Cristiana Lupi et al in their study they concluded that BRAF V600E mutation was found to be associated with follicular variant of papillary thyroid carcinoma with invasive tumor growth[33].

Other association between mutation and aggressive tumor phenotype include older age, extrathyroidal tumor invasion, lymph node and distant metastasis, higher tumor stage stage and poorly different cancer [34, 35].

However, in some study BRAF V600E mutation was found to be in patients with young age group [20].

F.Frasca et al [36] in their study found that presence of BRAF V600E mutation in PTC s is associated with aggressive tumor behaviour. They also found that the tumor aggressiveness is independently of tumor size suggesting that small BRAF positive tumors carry higher risk of progression and invasiveness than the BRAF negative tumors.

BRAF mutation can be readily tested on thyroid fine needle aspiration biopsy specimens, with high preoperative predictive probabilities for clinicopathological outcomes of papillary thyroid carcinoma[37].But it has limited diagnostic value because [29,38]of the low sensitivity of BRAF mutation when used in cytologically indeterminate specimens that are mostly non-PTC and therefore do not harbor BRAF mutation.

However controversies regarding BRAF mutations with poorer clinico pathologic outcome of papillary thyroid cancers have been reported in some studies [39, 40].

In thyroid, apart from papillary carcinoma BRAF mutation is also expressed in anaplastic carcinoma and poorly differentiated carcinoma and the prevalence is 20-30% and 10-15 % respectively. According to literatures [19] BRAF mutated poorly differentiated and anaplastic carcinoma will have

papillary component. This finding implies that these tumors may progress from BRAF positive papillary carcinoma.

BRAFV600E mutation is commonly not found in follicular thyroid cancer and benign thyroid nodules [32]. But BRAF K601E mutation was detected in follicular adenoma, carcinoma, and follicular variant of PTC. However Electron et al in their study they observed that BRAF V600E mutation was expressed in one case of follicular carcinoma [35].

With these as background we proposed to do a study in BRAF V600E mutation in various thyroid neoplasms at PSGIMS&R a tertiary care hospital, Coimbatore.

MATERIALS & METHODS

MATERIALS AND METHODS

All cases diagnosed as a thyroid neoplasm from Jan 2006 to Sep 2009 in the Department of Pathology, PSG Institute of Medical Sciences and Research, Coimbatore were considered for this study.

The clinical details of these cases were taken from the medical records department of PSG IMS&R, after obtaining permission from the authorities and due IHEC clearance. Age, sex, clinical presentation and hormone status were obtained by analyzing the case records. The T and N status of the malignant neoplasms was also noted for staging.

The H&E slides of all the cases were analyzed for the following: the type of neoplasm, various nuclear features, invasion into the capsule and vascular spaces, extra thyroidal extension, lymph node metastases, mitoses, necrosis and presence or absence of Amyloid. Paraffin blocks of those sections which had high tumor density with less normal thyroid tissue were included for the study by using H&E stained slides. Paraffin blocks of slides which showed tumor with large areas of hemorrhage, cystic change and necrosis were excluded from the study.

Two primers (Forward and Reverse primers) were designed to amplify a fragment of the exon 15 of BRAF containing site where the

V600E mutation occurs.Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (RFLP) method were carried out for mutation detection.

The following steps were followed in the methodology. (Fig 2)

STEP 1:

TISSUE PREPARATION FROM FORMALIN FIXED PARAFFIN EMBEDDED (FFPE) BLOCKS [38]

1) Using a pen, the area of the tissue containing the maximum tumor was marked on hematoxylin-eosin stained slide.
2) 10-15 μm sections were cut from formalin fixed paraffin embedded tissues and placed over the plain glass slide.
3) The sections were deparaffinized by immersion in xylene, followed by hydrated in graded alcohol
4) The H&E stained slide with the marked tumor area was kept over the unstained section slide.
5) Using the circled area of interest on the unstained tissue section slide as a guide, a clean scalpel blade was used to scrape the tissue in the area containing tumor tissue
6) The tissue was placed in a 2 ml of Eppendorf tube.

STEP 2:

ISOLATION OF DNA FROM FFPE

PROCEDURE: PHENOL CHLOROFORM METHOD

After placementment of tissue in Eppendorf tube, we dried the tissues at 50^{o}C, followed by we added 500 µL lysis buffer and 35 µL Proteinase K (20mg/mL) in each tube .Then the solution was incubated at 60^{o}C for 2-3 hours or until the tissue dissolves. Then we raised the temperature to 95^{o}C.

Inactivation of Proteinase K was done by incubating the tube for 8 min and then 15 µL RNase (10mg/mL) was added to each tube, followed by it kept it for incubation at 37^{o}C for 15 minutes.

Phenol: Chloroform (1ml) was added to each tube and then the solution was mixed properly. We centrifuged the mixture at 13,000 RPM for 5 minutes, then the supernatant was transferred to fresh tube then we added equal volume of choloroform and vortexed vigorously, again centrifuged at 13,000RPM for 5 minutes, supernatant was collected in a fresh tube .50 µL of 30M sodium acetate and 1mL of 100% ethanol added again, centrifugation was done at the same RPM and time as mentioned above and then the supernatant was discarded.

Finally the pellet was washed in 75% ethanol, followed by centrifugation at maximum RPM for 5-10 minutes was done. We dried the pellet and suspension of pellet in 35 µL of Milli Q.

The end product was run on an Agarose gel electrophoresis to check for the success of DNA isolation process (Fig 3).

STEP 3:

PCR OF ISOLATED DNA USING BRAF PRIMERS

PCR by using BRAF 15F and 15R primers was done to amplify a 224 bp fragment of the exon 15 of BRAF[39] containing the site in which V600E mutation occurs. PCR reaction were performed in 25 µl of 1.5 mM $Mgcl_2$ with 200µM deoxynucleoside triphosphates ,50-100 ng DNA,0.5 µM of each primer and 2.5 U Taq polymerase.

Forty cycles with annealing temperature optimized at 59 ^{o} C were used to apply PCR product .PCR amplification was confirmed on 2% Agarose gel (Fig 4) .The PCR product size was 224 bp.

STEP 4:

CLEAN UP OF PCR PRODUCT

PROCEDURE:

We used HIPURA-HIMEDIA clean up kit for this procedure.

1.5 ml of PCR product was taken in a tube and we added 4.5 volume of SBP binding solution (PCR binding solution), mixed thoroughly by gentle pipetting. Loading of lysate in Miniprep spin column followed by centrifugation at 10,000 x g for a minute at room temperature was done. We discarded the flow through.

Similar procedure was done by using 700µL of diluted wash solution (HPE) and 500 µL of diluted wash solution (HPE).Finally the empty tube was centrifuged for 2 minutes at 13,000 x g to dry column matrix, and then we transferred the column to new 2 ml tube, 30-50 µL of elution buffer was added. To elute DNA, centrifugation at 13,000 x g for 1 minute was done. Then we quantified the DNA by using Nanodrop.

STEP 5:

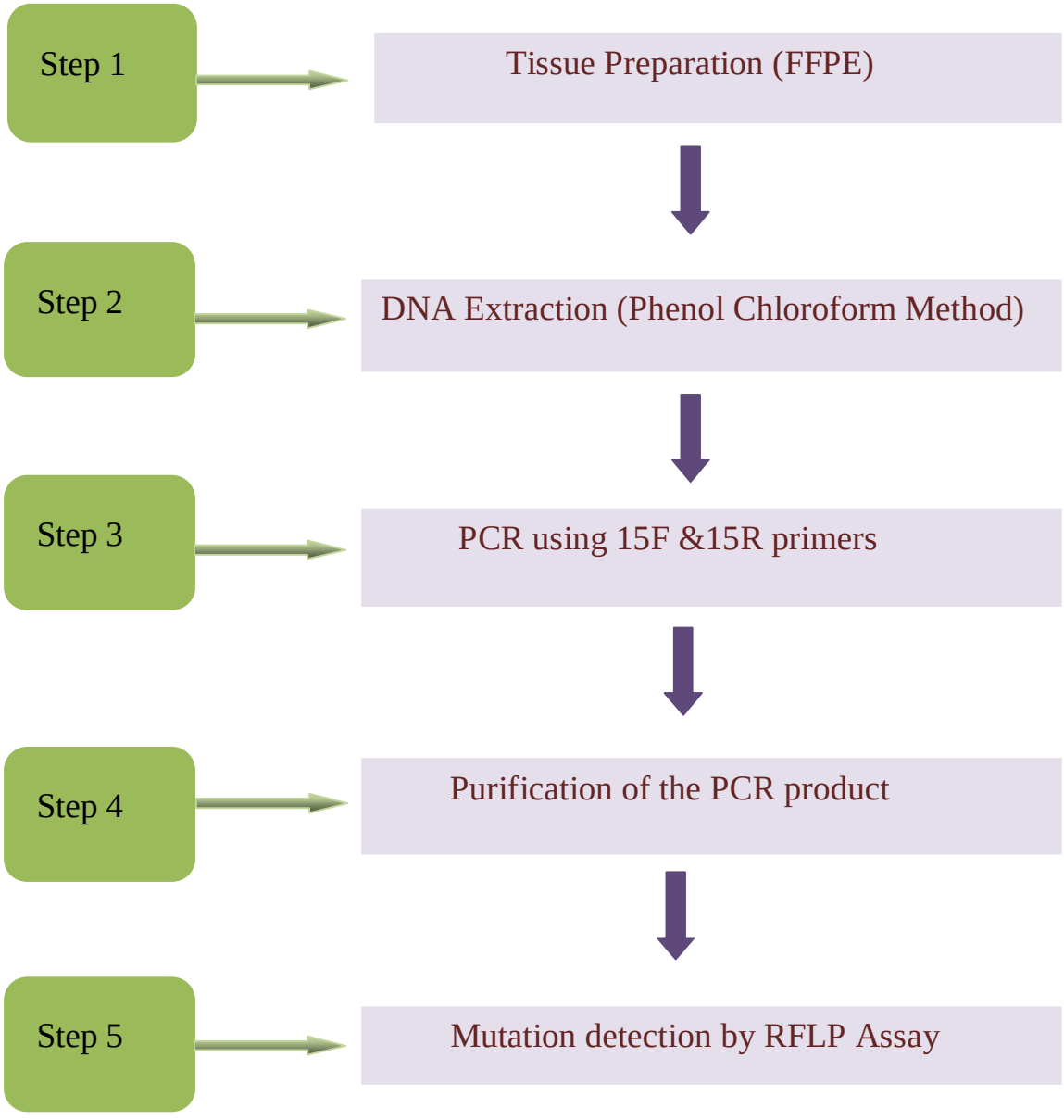
RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

RFLP was carried out by digesting the PCR product with TspRI fast digest (Fermentas). This enzyme digests the wild type alleles and the bands

are seen as 120 and 104 bp respectively. We incubated the product at 65°C for 1 1/2 hours and the product was checked in 12 % Polyacrylamide gel Electrophoresis (PAGE).

Polyacrylamide gel was run in Amersham electrophoresis system at 100 V for about 5 hours and the gel was stained with ethdium bromide.we viewed the stained gel in a Chemiluminescence gel documentation system to identify the DNA fragments. The wild type alleles are visualized as two bands digested by the enzyme. (Fig 5)

Fig 2: STEPS IN BRAF MUTATION ANALYSIS IN VARIOUS THYROID NEOPLASMS



FFPE – FORMALIN FIXED PARAFFIN EMBEDDED TISSUE; PCR-POLYMERASE CHAIN REACTION;

RFLP-RESTRICTION FRAGMENT LENGTH POLYMORPHISM.

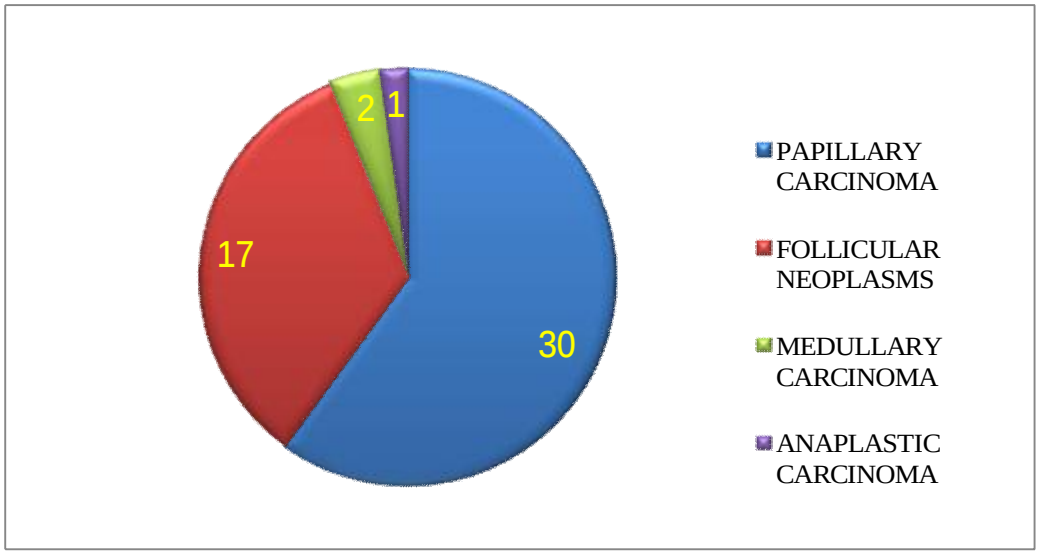
RESULTS

RESULTS

Department of pathology, PSGIMS&R received 15,739 biopsy specimens over a period of 3 years and 9 months (January 2006 to September 2009) of which 2953 were reported as malignant lesions. Of these 2953 malignancies 64 were thyroid neoplasms giving an overall incidence of 2.16%. 50 cases were selected from these 64 using the inclusion and exclusion criteria as mentioned before. The breakup of various types of thyroid neoplasms is as given in chart I.

CHART I: Types of Thyroid Neoplasms:

Total number of cases: 50



The age at presentation of these thyroid neoplasms ranged from 20-70 years as given in the Chart II below, with the mean age of 45 years. There

was a female preponderance of thyroid neoplasm reported as shown in Chart III.

CHART II. Thyroid Neoplasms- Age Distribution

Total number of cases: 50

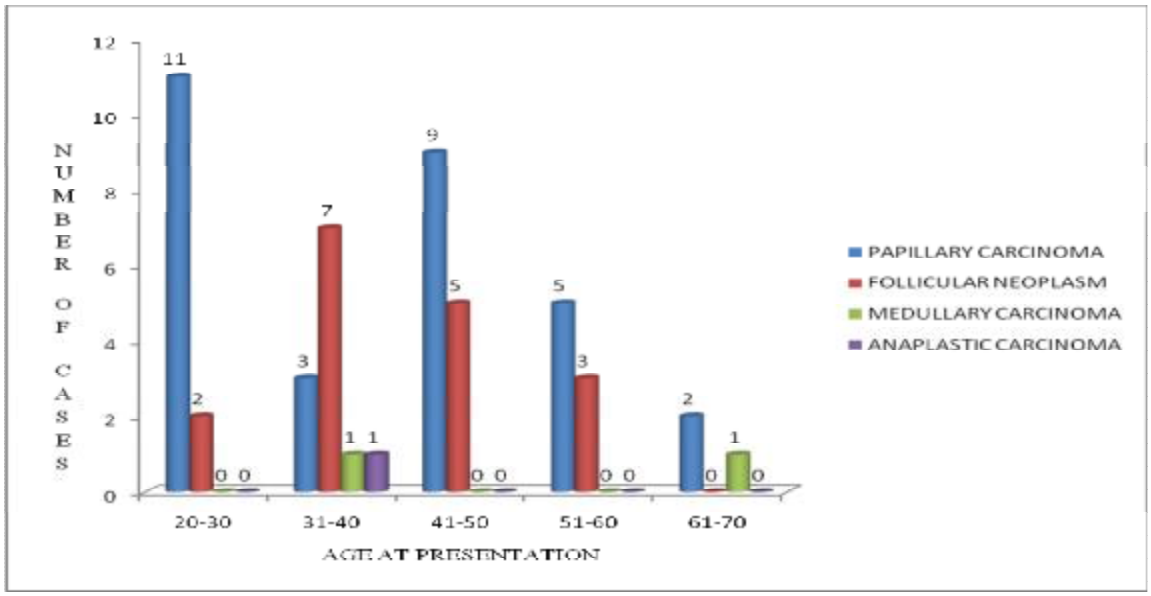
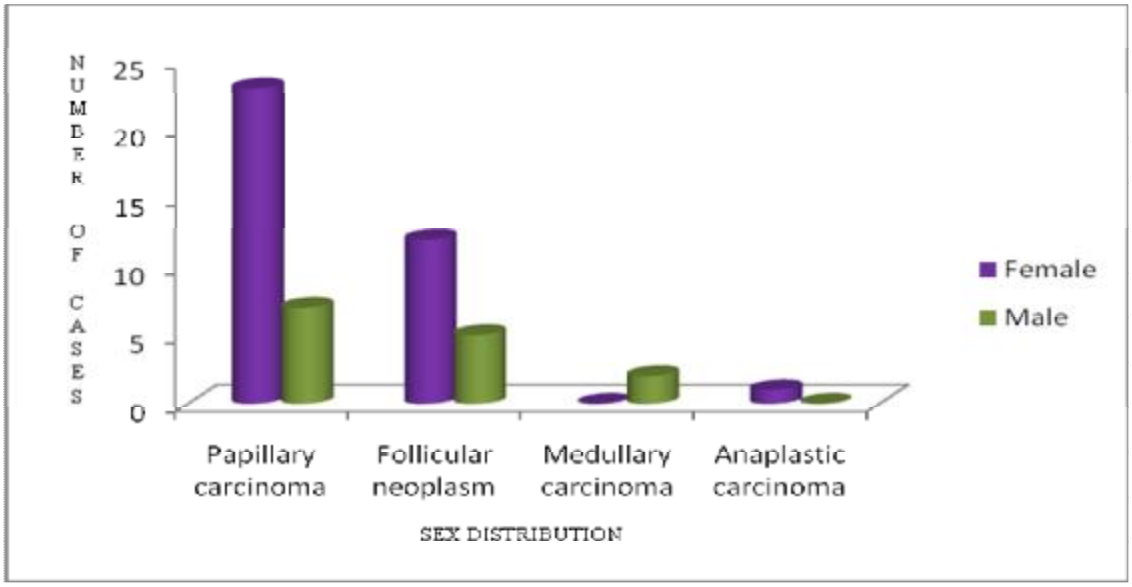


CHART III: Thyroid Neoplasms- Sex Distribution

Total number of cases 50



Among 50 case0s, 42 patients were in euthyroid state, 3 were hyperthyroid and 3 were in hypothyroid state and for 2 patients, hormone status was not available as shown in TABLE I.

TABLE I: Thyroid Neoplasms- Hormone Status

Hormone status	Euthyroid	Hypothyroid	Hyperthyroid	Not available	Total
No of cases	42	3	3	2	50

The gross features of these thyroid neoplasms studied are as given below.

The size of the tumor ranged from 0.4 to 11.5 cm and Table II shows the details of the size ranges and the types of thyroid neoplasms .The size of the tumour is essential for staging the neoplasms.

TABLE II: Thyroid Neoplasms-Size Variation

Size	Papillary carcinoma	Follicular neoplasm	Medullary carcinoma	Anaplastic carcinoma
<2cm	13	1	0	0
2 – 4 cm	13	10	2	0
>4 cm	3	6	0	1
Not assessed	1	0	0	0
Total (50)	30	17	2	1

Cystic changes were observed in 6 cases of papillary carcinoma. Multifocality was noted in 10 cases and 2 patients had lymph node involvement. Extrathyroidal extension was found in 2 cases.

All 17 cases of follicular neoplasms had a thick fibrous capsule around. Both the cases of medullary carcinoma had lymph node involvement. The lone case of anaplastic carcinoma measured 11.5 cm and involved right lobe and isthmus and also showed extrathyroidal extension.

H&E stained slides were analyzed for the following, microscopic features,

(i) Architectural pattern

(ii) Nuclear atypia

(iii) Vascular invasion

(iv) Capsular invasion

(v) Mitotic activity

(vi) Necrosis

(vii) Presence of Amyloid deposits in case of medullary carcinoma.

Among 30 cases of papillary thyroid carcinoma reported, 23 were papillary carcinoma classic type (Fig 6), 6 were micropapilary carcinoma (Fig 7) and the remaining one was encapsulated follicular variant of papillary carcinoma (Fig. 8) as shown in table III.

TABLE III: Thyroid Neoplasms – Papillary Carcinoma Variants

Papillary Ca	No of cases
Classic type	23
Micro carcinoma variant	6
Encapsulated Follicular variant	1
Total	30

Papillary thyroid carcinomas were also analyzed for the following microscopic features and scoring was done, according to Adebowale J.Adeniran et al [41].

1. Nuclear enlargement
2. Irregularity of nuclear membrane
3. Chromatin clearing
4. Nuclear crowding /overlapping
5. Nuclear grooves
6. Nuclear pseudoinclusions
7. Fibrosis
8. Psammoma bodies
9. Inflammatory response

The presence of first five features in the sections confined the specimens into the following scoring categories. If none of the features were observed the score given is 1, if <10 % of the section studied show above features the score is 1+, if the section studied revealed 10-50% or >50% of the above features the scoring was graded as 2+ and 3+ respectively.

The presence of pseudoinclusions were scored as given below .Score 0-No pseudoinclusions /10HPF, Score 1+-1-2 pseudoinclusion /10HPF, Score 2+ - 3 to 5 pseudoinclusion /10HPF and Score 3+ - >5 pseudoinclusions found in 10 high power fields.

Presence of tumor fibrosis was scored as 0, 1+, 2+, and 3+ for none, mild, moderate and severe respectively. Presence of inflammatory response (Chronic inflammatory cell aggregates) and Psammoma bodies were counted for 10 HPF was scored as 0, 1+ , 2+ and 3+ for 0,1,2 to 5, and > 5 respectively and was correlated with BRAF positive cases as shown in table

Out of 17 cases of follicular neoplasms, 13 cases were folicular adenoma and 4 were follicular carcinoma. Of the 13 follicular adenomas, 11 were usual type (Fig 9), 1 was signet ring cell type (Fig 10) , and the remaining one was follicular adenoma with papillary architecture. Among 4 cases of follicular carcinoma, 2 were minimally invasive (Fig 11) , one was

Hurthle cell neoplasm (Fig 12) with focal capsular invasion and one was follicular neoplasm of uncertain malignant potential. (Fig 13)

TABLE IV : Follicular neoplasm and its variants.

Type of neoplasm		No of cases
Follicular adenoma n=12	Usual type	11
	Micro follicular adenoma- signet ring cell type	1
	Follicular adenoma with papillary architecture	1
Follicular carcinoma n = 4	Minimally invasive follicular carcinoma	2
	Hurthle cell neoplasm with capsular invasion	1
	Follicular neoplasm of Uncertain malignant potential	1

Both cases of medullary carcinoma showed the cells in nests and sheets (Fig 14)along with amyloid in the stroma (Fig 15), as proven by congored staining and by polarized microscopy.The single case of Anaplastic carcinoma of thyroid showed solid pattern of arrangement of cells with marked pleomorphism , increased mitotic rate,necrosis and evidence of invasion into the adjacent muscle fibers.(Fig16)

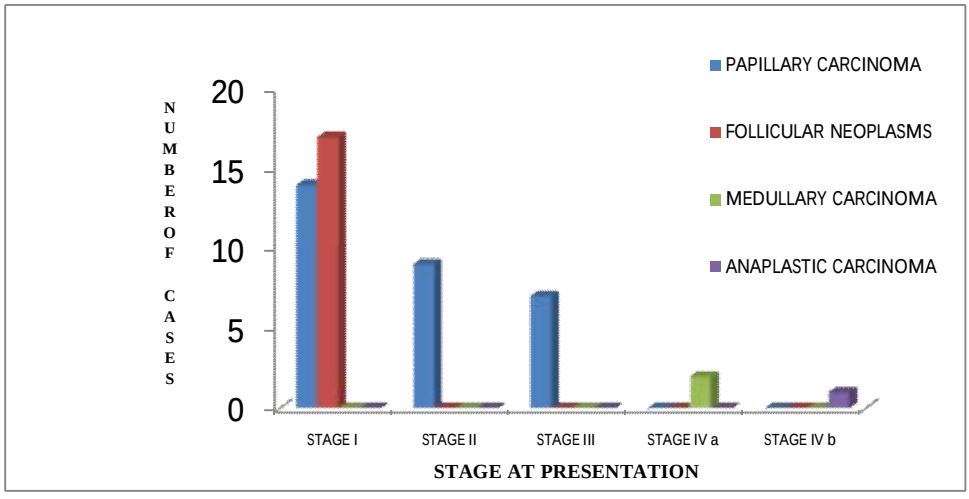
Staging of these was done according to TNM classification and is as given in the table V and chart IV.

TABLE V: Thyroid Neoplasms - Stage at Diagnosis

Stage	Papillary carcinoma	Follicular neoplasms	Medullary carcinoma	Anaplastic carcinoma
I	14	17	0	0
II	9	0	0	0
III	7	0	0	0
IVa	0	0	2	0
IVb	0	0	0	1
Total (50)	30	17	2	1

CHART IV: Thyroid Neoplasms - STAGE AT DIAGNOSIS

Total number of cases 50



BRAF MUTATION - RESULTS:

Out of 50 cases studied, DNA could be extracted from 47 cases only. DNA extraction was not possible for the 3 cases. Of these 47 cases, 14 cases expressed BRAF mutation. Among these 14, 9 were papillary carcinomas and 5 were follicular neoplasms. Out of 5 positive follicular neoplasms, 3 were follicular adenoma and the other 2 were follicular carcinoma as shown in table VI.

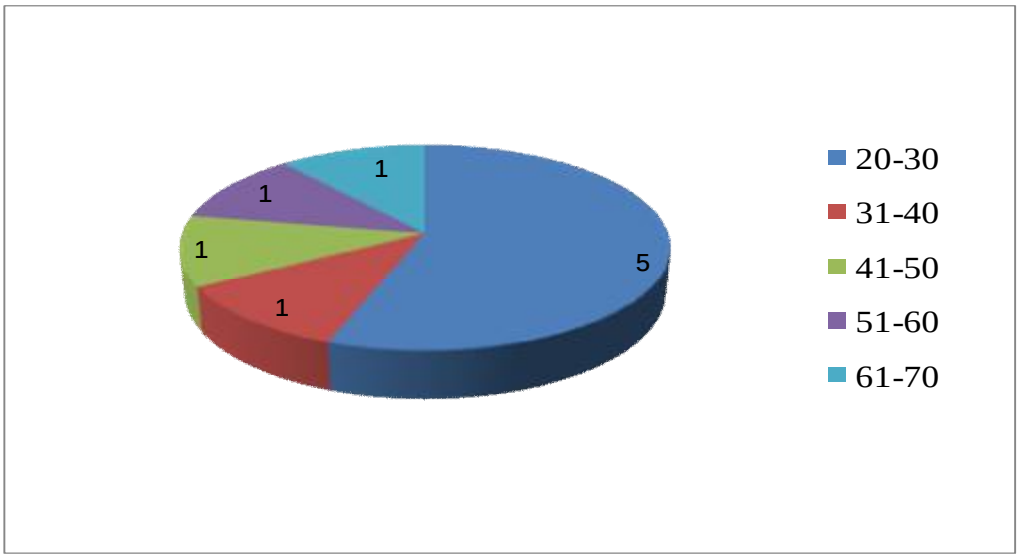
TABLE VI: BRAF V600E Positive Cases and Type Of Thyroid Neoplasms

Papillary carcinoma		**Folicular neoplasm**		**Total**
Conventional type	Encapsulated follicular variant	Follicular Adenoma	Follicular Carcinoma	
8	1	3	2	14

The BRAF V600E mutated cases were analyzed with various phenotypic features and the results are given in chart V, VI and VII and in table VII.

Out of 9 cases of BRAF positive papillary carcinomas, 5 cases were between the age group of 20-30 yrs and one case each in the 3^{rd}, 4^{th},5^{th} and 6^{th} decade.

CHART V : Number of BRAF Positive Papillary Thyroid Carcinoma - Age Distribution n=9



Out of the 9 cases of BRAF positive papillary thyroid carcinomas 8 were females as shown in TABLE VII.

TABLE VII: Carcinoma - Sex Distribution

Sex	BRAF Positive PTC
Male	1
Female	8
Total	9

Out of 9 patients 8 patients were in euthyroid state (88.88%) and 1 in hypothyroid state as shown in Chart VI. 55.55% of BRAF V600E positive

cases found to be in stage I(5/9 cases), stage II was present in 3 cases (33.33%) and 1 case was found in stage III as given in chart VII.

CHART VI: Number of BRAF Positive Papillary Thyroid Carcinoma- Hormone Status

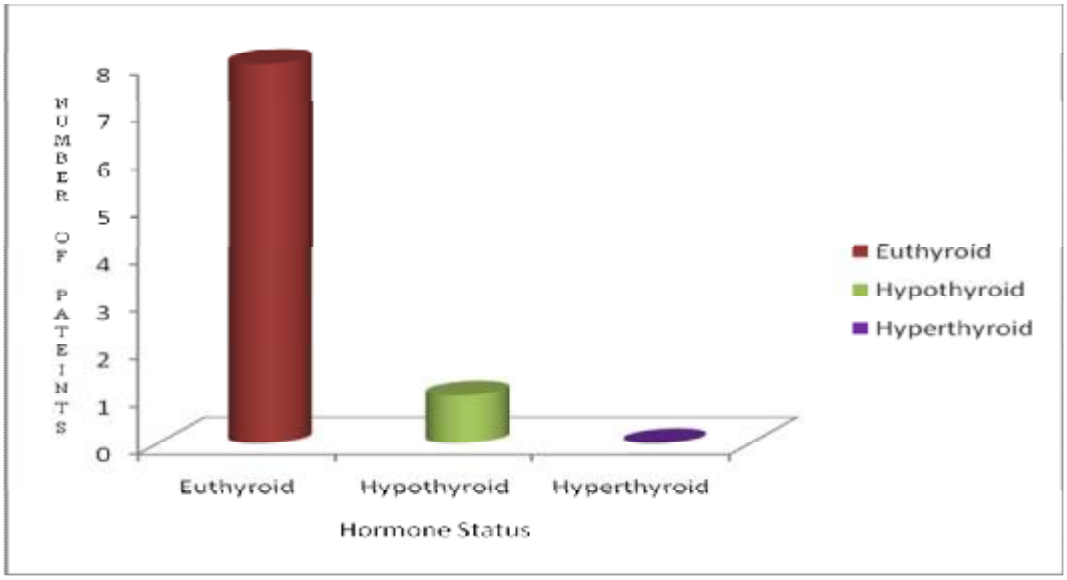
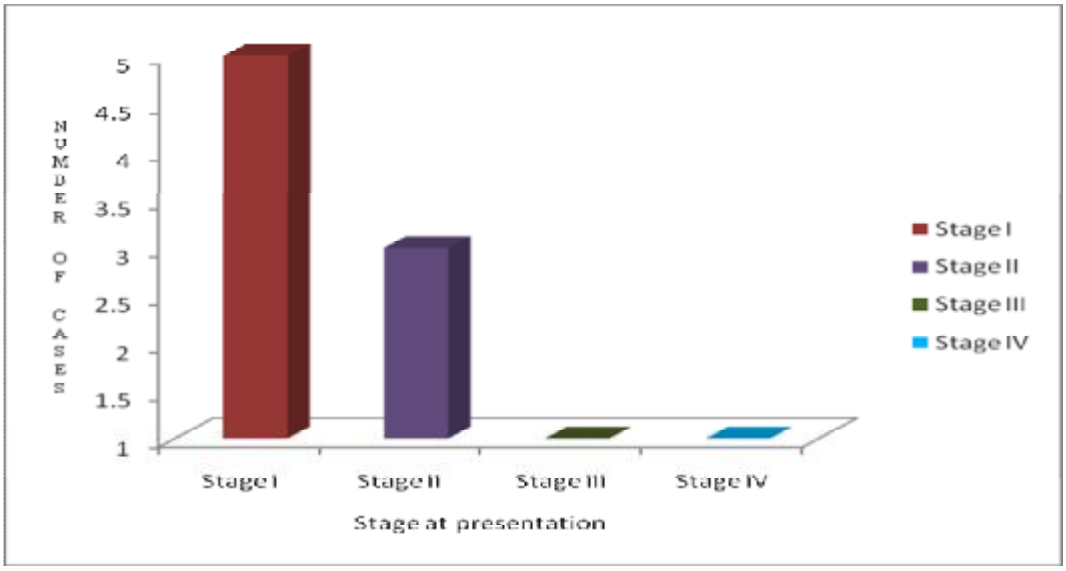


CHART VII: Number of BRAF Positive Papillary Thyroid Carcinoma - Stage at Presentation



Scoring for nuclear features and other microscopical features revealed consistent nuclear features in BRAF positive Papillary Carcinoma s as shown in Table VIII

TABLE VIII: Scoring for nuclear and other microscopical feature - BRAF positive papillary carcinoma

S. No	NE	NI	CC	NC	NG	PI /10HPF	Fib	PB /10HPF	Inflammatory Cell aggregate /10HPF
1	1	1	2	3	3	0	1	2	1
2	2	1	3	3	2	0	0	0	0
3	2	1	3	3	2	1	1	0	0
4	2	2	1	3	2	2	1	0	0
5	2	1	3	2	2	1	1	0	0
6	2	1	3	2	1	0	2	2	0
7	2	0	1	3	2	0	0	0	0
8	2	0	1	2	3	0	0	0	0
9	3	3	1	1	2	2	3	3	3
Average score	2	1.1	2	2.4	2.1	0.6	1	0.7	0.4

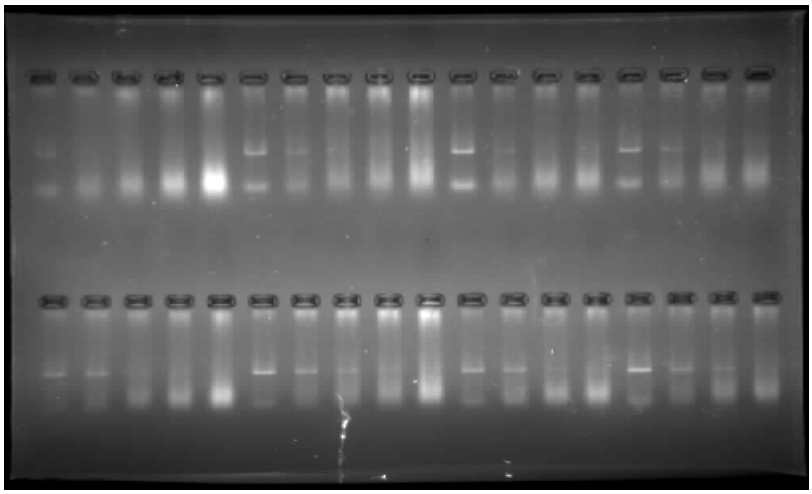
NE-Nuclear Enlargement ; NI- Nuclear Irregularity;CC-Chromatin Clearing;NC-Nuclear Crowding;NG-Nuclear Grooves; PI-Nuclear Pseudoinclusion;Fib-Fibrosis;PB-Psammoma Bodies.

TABLE IX: Distribution of BRAF mutation among different types of Thyroid Neoplasms

Types of neoplasms	BRAF V600E positive (%)	Wild Type (%)	Total
Papillary Carcinoma	9 (31)	20 (69)	29
Follicular Neoplasm	5 (31.25)	11 (68.75)	16
Medullary Carcinoma	0	1 (100)	1
Anaplastic Carcinoma	0	1 (100)	1
Total	14 (29.8)	33 (70.2)	47

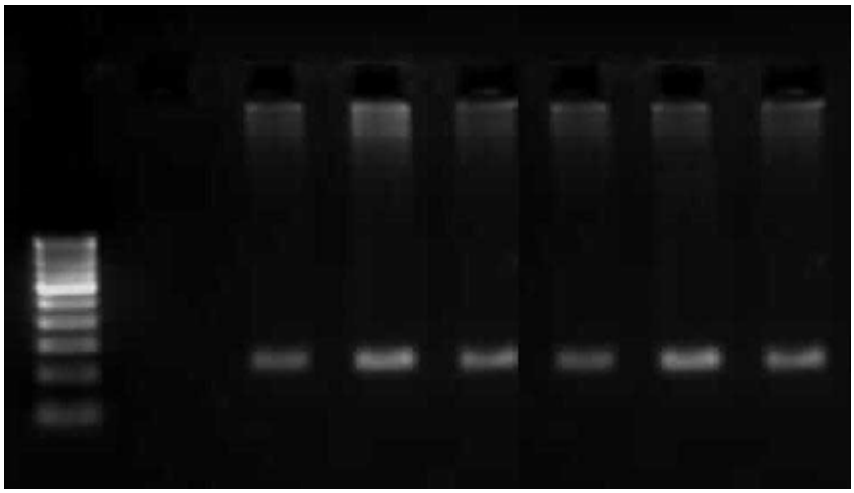
The above table depicts the distribution of BRAF V600E mutation among different types of thyroid neoplasms.

Fig 3: Agarose Gel Electrophoresis: DNA EXTRACTION



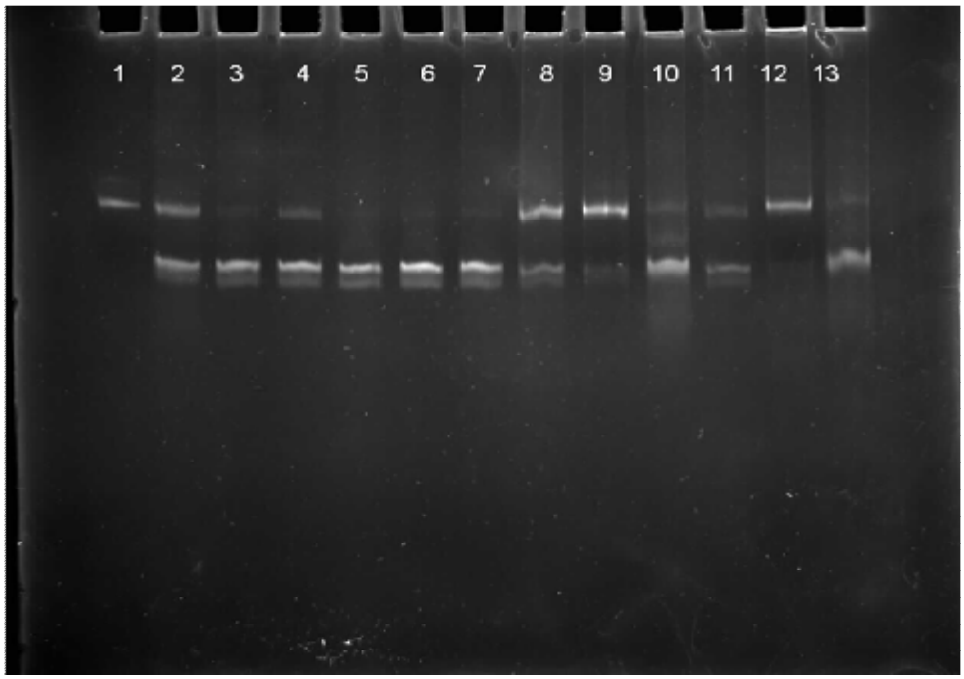
Extraction of DNA from Formalin Fixed Paraffin Embedded tissue .Run on Agarose gel and was confirmed with a gel doc. Photo which shows the band confirming the successful DNA isolation.

Fig 4: Agarose Gel Electrophoresis: PCR AMPLIFICATION



Gel electrophoresis image of PCR amplified product, by using 15F and 15R primers. The product size is 224bp.

Fig 5: Poly Acrylamide Gel Electrophoresis : RESTRICTION DIGESTION



Control: Well No - 1 (224bp)

Wild type: Well No - 3, 5, 6, 7, 13 (120bp+ 104bp)

Mutant: Well No - 2, 4, 8, 9, 10, 11, 12 (224bp and 224+120+104bp)

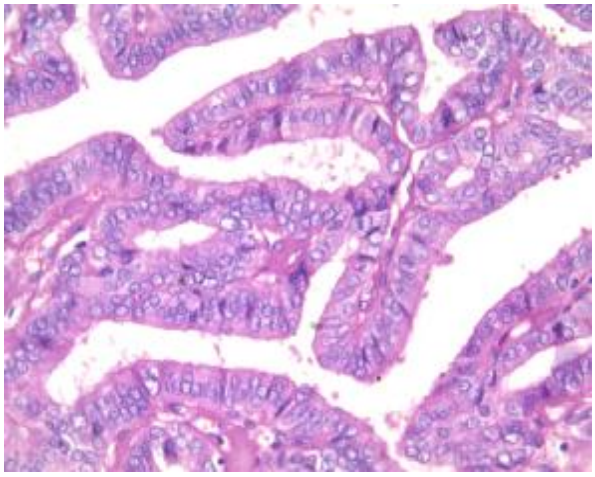


Fig 6 : Nuclear features of Conventional Papillary carcinoma. H & E (x400)

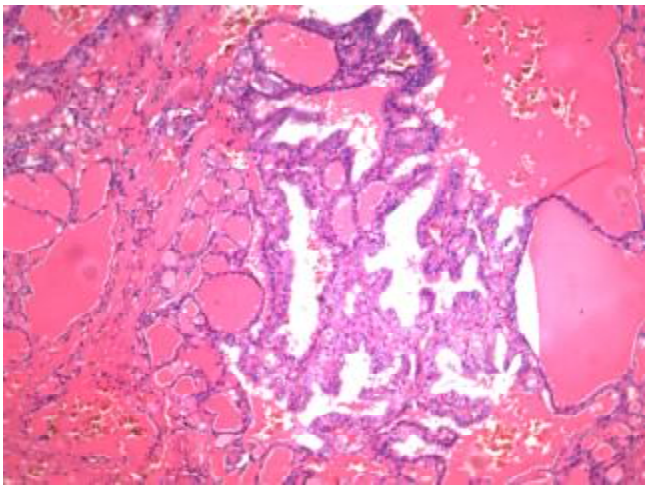


Fig7: Micro papillary carcinoma surrounding normal thyroid tissue. H&E(x100)

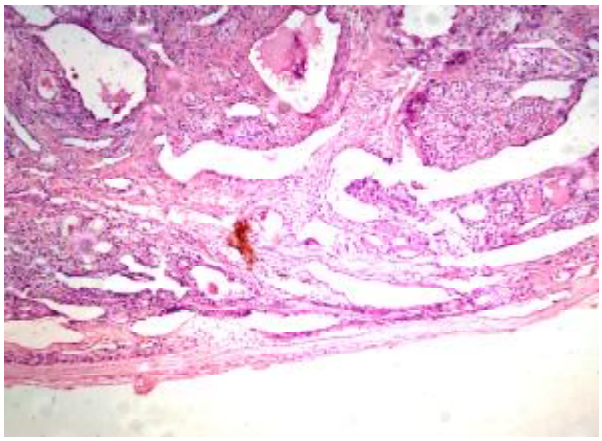


Fig 8: Encapsulated follicular variant of Papillary Carcinoma with thin capsule and cells in follicular pattern. H & E (x100)

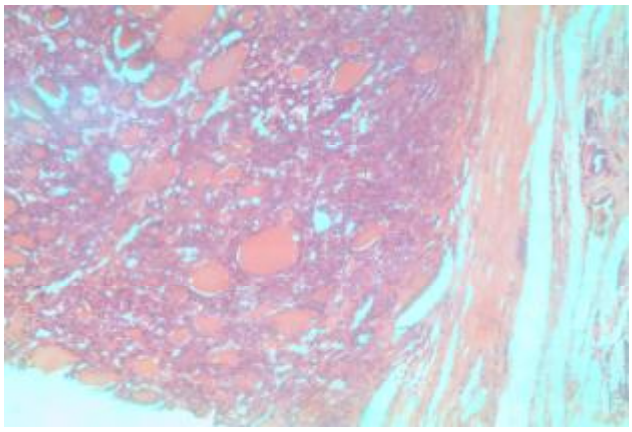


Fig 9: Follicular adenoma with a thick capsule H & E (x100)

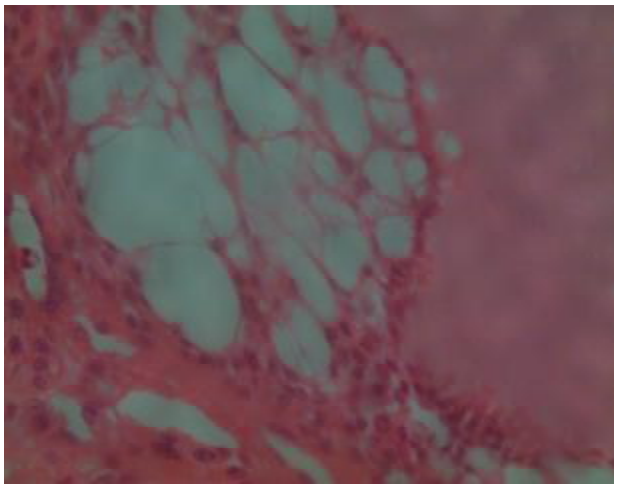


Fig 10: Signet ring cell type follicular adenoma. H & E (x400)

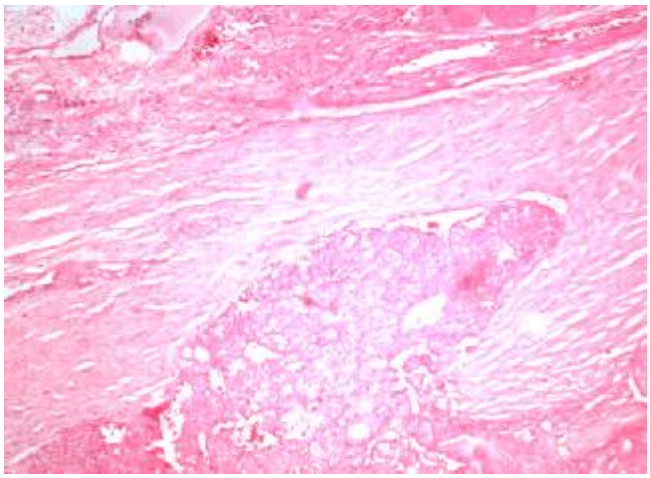


Fig 11 : Follicular neoplasm with minimal capsular invasion . H & E (x100)

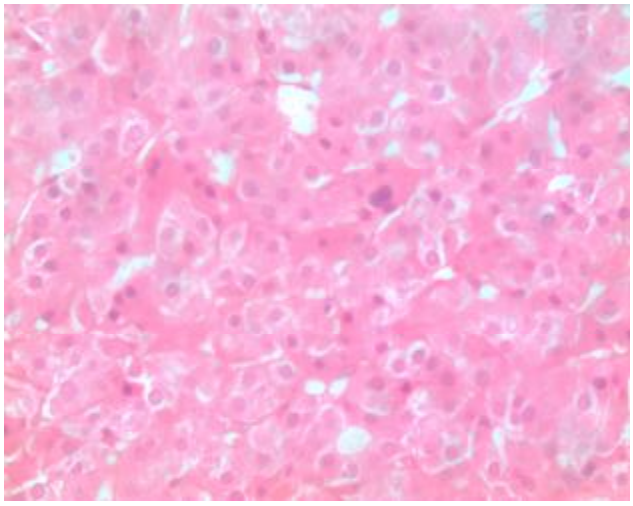


Fig 12: Hurthle cell neoplasm H & E (x400)

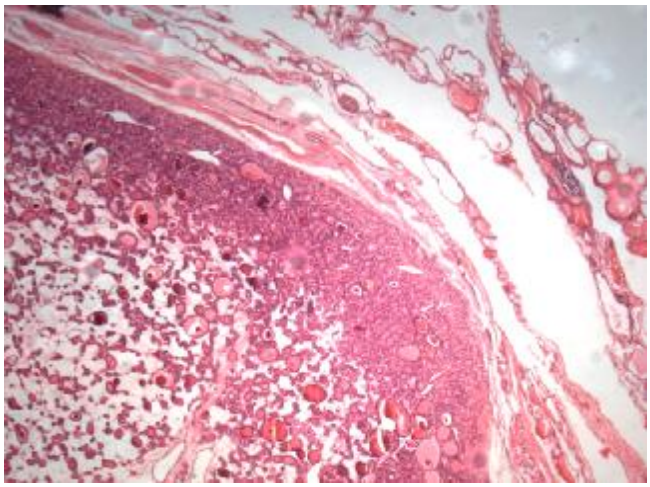


Fig 13: Follicular neoplasm uncertain malignant potential (x100)

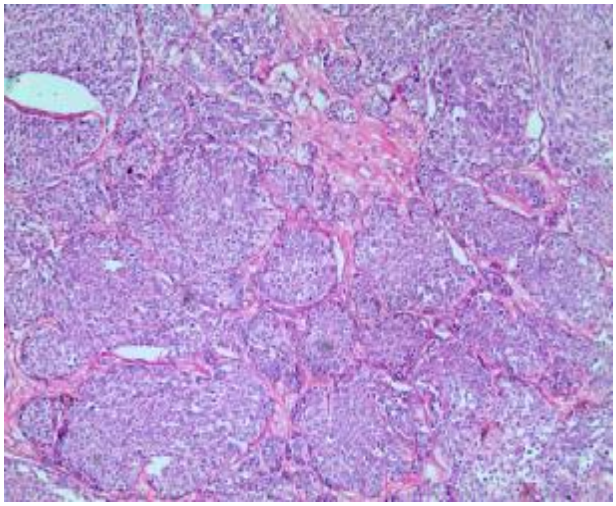


Fig 14:Medullary carcinoma H&E (X100)

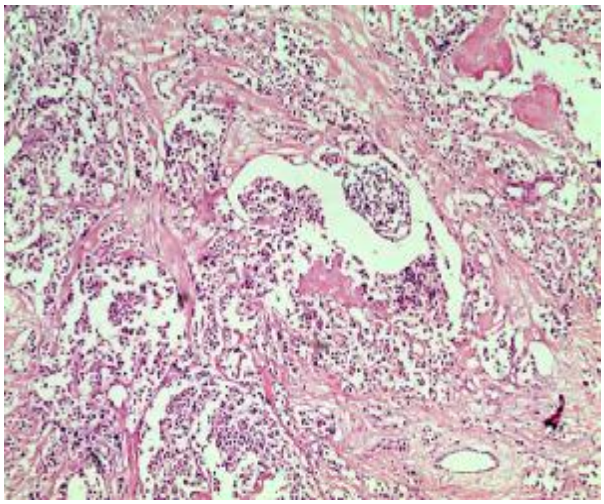


Fig 15:Medullaery carcinoma showing amyloid deposits in the stroma .H & E (x100)

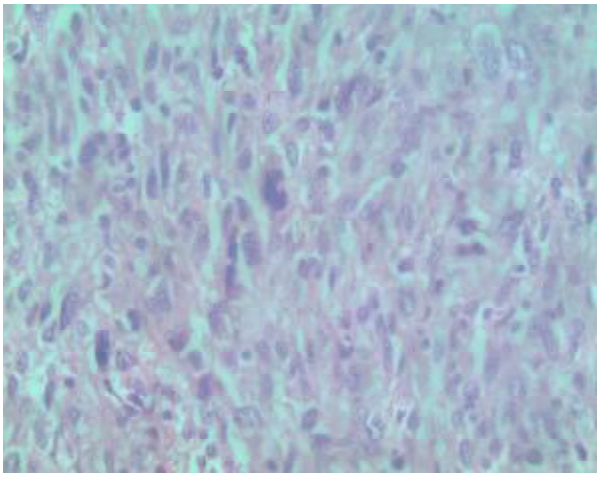


Fig 16.Anaplastic carcinoma H & E (x400)

DISCUSSION

DISCUSSION

Malignancies constituted 19.2% among all the biopsies reported at PSGIMSR during the study period, similar to the incidence reported from Chennai cancer registry [42]. The thyroid neoplasms constituted 2.16% of all malignancies (64 over 2953) in this institute and are similar to Chennai Cancer Registry reports [42] while it is higher than the 1% incidence reported in the Western countries [12].

Papillary thyroid carcinomas constituted around 64% of thyroid neoplasms similar to the incidence reported from Chennai cancer registry (50-90 %).

A report from teaching hospital, Chennai states that the incidence of papillary thyroid carcinoma is common in coastal areas [43]. Coimbatore is situated in the western part of Tamilnadu, and is a non-coastal, iodine deficient area as reported by a population survey conducted in 1991, by the Tamilnadu health and family welfare. However, the population of Coimbatore receives dietary iodine supplementation in the form of iodized salt from 1994[8]. A population survey indicates that high dietary iodine is associated with a high risk of thyroid neoplasms especially papillary carcinoma of thyroid [9]. Given the above observations, it is important to

plan further studies to estimate average population iodine levels in Coimbatore.

Further, BRAF V600E mutation is associated with papillary thyroid carcinomas. Identification of this mutation has changed the algorithm of treatment approach to papillary thyroid carcinomas. Some of the prevailing local factors indicated by our biopsies including the increasing incidence, female preponderance, younger age at presentation and an increase in incidental finding of papillary carcinoma in thyroidectomy specimens along with the potential for altering the treatment approach to these patients, prompted us to do this study.

An extensive literature search on Indian studies for BRAF mutation in thyroid carcinoma indicated a paucity of literature. For example, a Pubmed search (from1960-2010) with MESH keywords of “BRAF” and “thyroid neoplasm” and “India” returned no reports. Thus, we considered it to be important for us to do this study.

We identified and retrieved paraffin blocks of 50 cases of thyroid neoplasms from archives of pathology using exclusion and inclusion criteria as discussed earlier. We could extract DNA from 47 cases only. DNA extraction was not possible for 3 cases (one each of papillary carcinoma,

follicular adenoma and medullary carcinoma) probably due to improper fixation.

BRAF V600E mutation when present in papillary carcinoma of thyroid indicates a poorer outcome of the disease [26, 27]. Studies also state that, though this mutation can be seen in early stages, this is most often expressed in stage III (30%) when compared to Stage I. The expression of BRAF mutation in early stages indicates a possible aggressive course and warrants total thyroidectomy .When present in later stages of the disease, this is associated with a poorer outcome.

In our study group of 30 papillary carcinomas, DNA was extracted from 29 cases. 9 cases were positive for BRAF V600E mutation. Hence 31% (9/29cases) of papillary carcinoma thyroid expressed BRAF V600E mutation. Literature search states that BRAF V600E mutation is reported in 40-45% of papillary thyroid carcinomas [22] and we infer from our study that there is a lesser incidence of V600E mutation expression in papillary thyroid carcinomas in the subset of population that received treatment in our centre .But interestingly we found BRAF V600E mutation in 31% of cases of follicular neoplasms, which is assessed and discussed further down.

Out of 9 cases expressing V600E mutation 5 cases were in the age group between 20-30 years. Rossella et al [5] states that V600E is expressed

in papillary carcinoma occurring in older age group. In our study this is seen in a younger age group. BRAF V600E is known to induce a more aggressive phenotype and therefore this may have resulted in clinical expression in younger age group. As V600E mutation is also associated with an aggressive course, total thyroidectomy is warranted in cases expressing V600E in early age group. Therefore surveillance for mutation especially on FNA samples will prevent more repeat surgeries and help in better follow-up care of the patient.

5 cases of stage I, 3 cases of stage II and 1 case of stage III expressed V600E mutation. The analysis of our study shows a significantly higher expression in stage I and stage II lesions. Therefore we reanalyzed the reports of papillary thyroid carcinoma. As we are in a tertiary care hospital with advanced facilities for patient care, radiologist and pathologist were probably able to detect papillary thyroid carcinoma in early stages, thereby explaining the higher incidence of V600E mutation in stage I papillary thyroid carcinomas.

There was no significant correlation with the nuclear features and BRAF V600E mutation but nuclear crowding, overlapping and grooves were consistent finding (Table VIII).

As previously noted, 5 cases of follicular neoplasm expressed BRAF V600E mutation with a percentage incidence of 31% .Literature survey states that V600E mutation is not commonly seen in follicular neoplasms and a few studies quote no more than 1% incidence of this mutation in follicular neoplasms [35, 44]. Therefore reanalysis of routine H&E slides were done and 2 cases had focal papillary architecture with nuclear features which might have been overlooked in morphological assessment .Excluding these 2 cases the percentage incidence of V600E mutation in follicular neoplasm is 18.75%.As bits are given only from the representative areas and are not sampled extensively on a routine basis, it is likely that papillary carcinoma especially micro follicular type could have been missed.

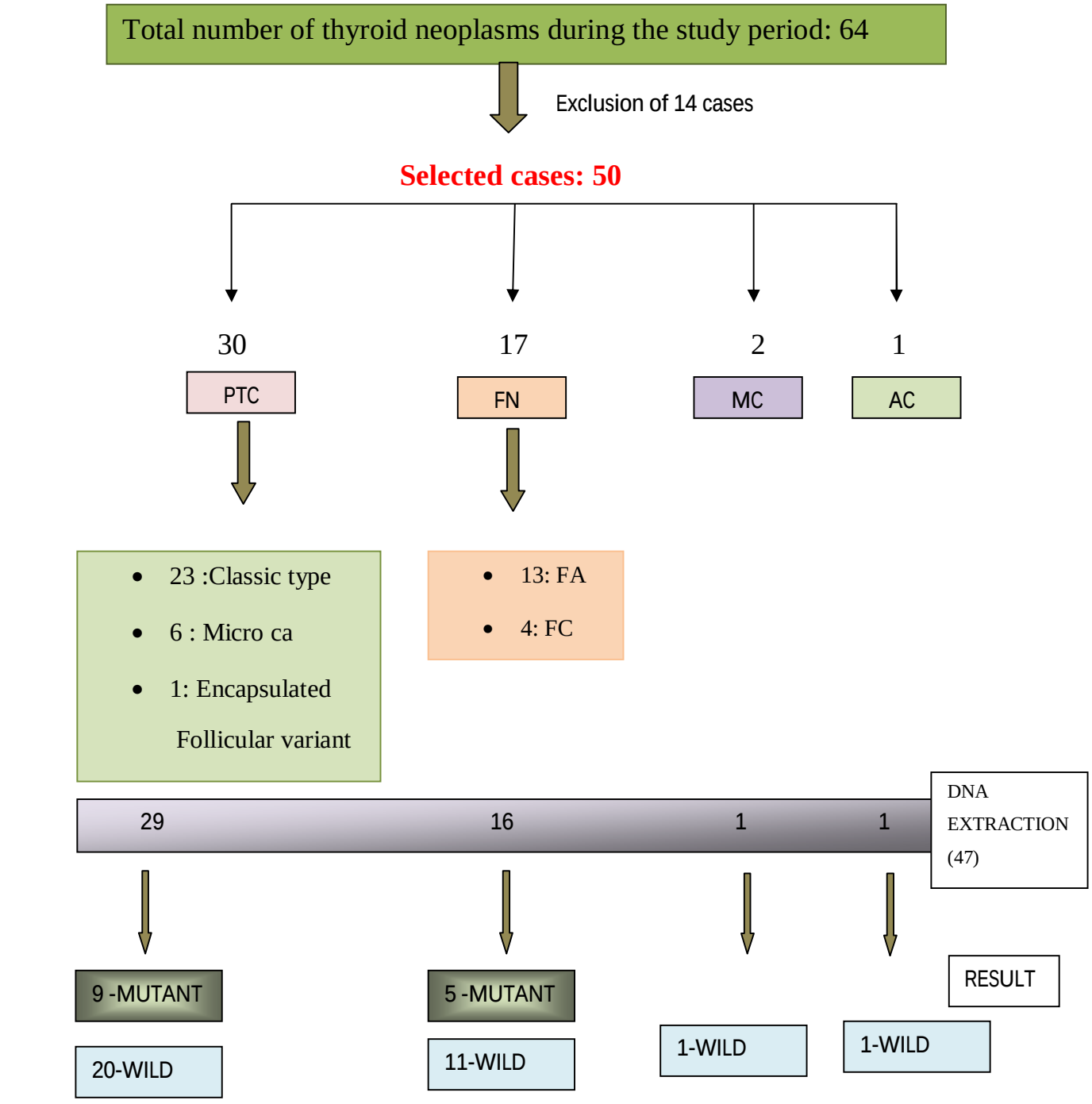
Therefore from our study, we infer that a significant number i.e., 31% of papillary carcinoma (9/29) and 18.75% of follicular neoplasms (3/16) express V600E mutation. This mutation was expressed more in stage I of papillary thyroid carcinoma. As BRAF mutation is associated with aggressive behavior, a close follow up and total thyroidectomy becomes essential. If mutational studies are done on cytology specimens prior to surgery, there might be a reduction in number of repeat completion thyroidectomy procedure as total thyroidectomy is treatment of choice in BRAF positive cases. It might also be useful to do BRAF mutation studies

where there are no firm diagnosis possible by cytology. A larger prospective study including cytology specimen prior to surgery and surgical resection specimens of the same patients is essential to prove the utility of V600E mutation as a routine diagnostic tool. Once developed the primers become cost effective to the patient. Also as targeted therapies are available, mutational studies might be the future diagnostic methods of these tumors.

It has been demonstrated that there is an increase in the incidence of papillary carcinoma where there is high iodine intake [3, 7, 15] either in diet or in the form of iodine supplementation in a population where there is an existing V600E mutation. This raises the question over routine high iodine supplementation for preventing non-neoplastic conditions such as multinodular goiter. A larger multicentric, prospective study addressing this might prove to be useful.

As our study showed an increased expression of BRAF V600E mutation in follicular neoplasms, a larger study of V600E mutation in follicular neoplasms is vital to look for a change in pattern of expression of mutational status in Indian population. Algorithm of our approach to this study is given in Fig 17.

Fig 17: **Algorithm of the study**



PTC- Papillary Thyroid Carcinoma ; FN- Follicular Neoplasm ; FA- Follicular Adenoma; FC-Follicular Carcinoma; MC-Medullary Carcinoma ; AC-Anaplastic Carcinoma

SUMMARY & CONCLUSION

SUMMARY AND CONCLUSION

In conclusion, our study on thyroid specimens reported at PSGIMS&R from January 2006 to September 2009 revealed a 31% incidence of papillary carcinoma and 18.75% incidence of follicular neoplasms expressing BRAF V600E mutation. We also infer that BRAF V600E mutation was seen more in stage I and therefore a close follow up or completion thyroidectomy becomes essential. The limitation of our study includes small sample size. Therefore a larger multicentric study prospective study on both FNA and surgical specimen with long term follow up is essential to prove the utility of BRAF V600E mutational study as a routine diagnostic tool for treatment and prognostication.

Probably ,epidemiologic survey of iodine levels and V600E mutation in patients with multinodular goiter and cases where incidental papillary thyroid carcinoma reported might go a long way in developing screening modalities for thyroid neoplasms and prevention of this increasing incidence of thyroid neoplasms.

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MASTER CHART

MASTER CHART

S. No	HP. No	Age/ Sex	Hormone Status	Size	Other features	Diagnosis	Stage	BRAF
1.	150/06	30/M	Euthyroid	2.6cm	-	Minimally invasive follicular carcinoma	I	Mutant
2.	458/06	48/F	Euthyroid	0.4cm	-	Papillary microcarcinoma	I	Mutant
3.	747/06	49/F	Euthyroid	3.5cm	Multifocal, cystic	Papillary carcinoma	II	Wild
4.	888/06	50/F	Euthyroid	NA	Extrathyroidal extension	Papillary carcinoma	III	Wild
5.	1253/06	51/F	Hyperthyroid	0.5cm	Multifocal	Papillary microcarcinoma	I	NE
6.	1562/06	32/F	Euthyroid	2.3cm	-	Follicular adenoma	I	Wild
7.	1801/06	38/M	Euthyroid	1.8cm	Lymph node involvement	Medullary carcinoma	IV A	Wild
8.	1906/06	48/M	NA	4.2cm	-	Follicular adenoma	I	NE
9.	2093/06	23/F	Euthyroid	5.5cm	Multifocal	Papillary carcinoma	III	Wild
10.	2110/06	43/M	Euthyroid	2.5cm	-	Follicular adenoma with papillary architecture	I	Mutant
11.	2230/06	57/M	Euthyroid	3.0cm	-	FN- UMP	I	Wild
12.	2232/06	58/M	Euthyroid	7.0cm	-	Microfollicular adenoma-signet ring cell type	I	Mutant
13.	2780/06	52/F	Euthyroid	0.4-2.3cm	Multifocal	Papillary carcinoma	II	Mutant

14.	2838/06	35/F	Euthyroid	9.5cm	-	Follicular adenoma	I	Wild
15.	3173/06	34/F	Euthyroid	3.0cm	-	Follicular adenoma	I	Wild
16.	29/07	51/F	Euthyroid	3cm	Cystic	Papillary carcinoma	II	Wild
17.	220/07	70/M	Euthyroid	2.0-4.0cm	Multifocal	Papillary carcinoma	II	Wild
18.	307/07	38/M	Euthyroid	0.5cm	-	Papillary microcarcinoma	I	Wild
19.	600/07	67/M	Hypothyroid	5.5cm	Lymph node involvement	Medullary carcinoma	IV A	NE
20.	1310/07	43/F	Euthyroid	3.3cm	-	Follicular adenoma	I	Wild
21.	1560/07	50/F	Euthyroid	0.3-1.0cm	Multifocal, cystic	Papillary carcinoma	I	Wild
22.	1572/07	30/F	Euthyroid	0.4cm	-	Papillary microcarcinoma	I	Wild
23.	1651/07	36/F	Euthyroid	5.5cm	-	Follicular adenoma	I	Wild
24.	2118/07	45/F	Euthyroid	2.5cm	-	Follicular adenoma	I	Wild
25.	2710/07	34/F	Euthyroid	3.0cm	-	Follicular adenoma	I	Wild
26.	2874/07	65/F	Hypothyroid	1.3cm	-	Papillary carcinoma	I	Mutant
27.	3335/07	45/F	Euthyroid	6.0cm	-	Hurthle cell neoplasm	I	Mutant

28.	3478/07	35/F	Hyperthyroid	4.5cm	-	Follicular adenoma	I	Wild
29.	3679/07	27/F	Euthyroid	3.0cm	-	Papillary carcinoma	II	Wild
30.	3693/07	46/F	Euthroid	0.8-5.0cm	Multifocal	Papillary carcinoma	III	Wild
31.	648/08	55/F	Euthyroid	1.0cm	-	Follicular adenoma	I	Wild
32.	1101/08	30/F	Hypothyroid	3.0cm	-	Minimally invasive follicular carcinoma	I	Wild
33.	1376/08	24/F	Euthyroid	5.5cm	Cystic	Papillary carcinoma	III	Wild
34.	1422/08	53/M	Euthyroid	2.0cm	-	Papillary carcinoma	I	Wild
35.	1837/08	30/F	Euthyroid	0.3cm	-	Encapsulated follicular variant of Papillary carcinoma	I	Mutant
36.	1865/08	29/M	Euthyroid	2.5cm	-	Papillary carcinoma	II	Mutant
37.	2062/08	35/F	Euthyroid	4.0cm	-	Follicular adenoma	I	Mutant
38.	2222/08	27/F	Euthyroid	2.4cm	-	Papillary carcinoma	II	Mutant
39.	2850/08	25/M	Euthyroid	0.5cm	-	Papillary microcarcinoma	I	Wild
40.	3187/08	27/F	Hyperthyroid	0.5cm	-	Papillary microcarcinoma	I	Wild
41.	4248/08	32/F	Euthyroid	1.5cm	-	Papillary carcinoma	I	Wild

42.	011/09	45/F	Euthyroid	3.5cm	-	Papillary carcinoma	II	Wild
43.	229/09	28/F	Euthyroid	0.3, 1.4cm	Multifocal, cystic	Papillary carcinoma	I	Mutant
44.	824/09	41/F	Euthyroid	0.3-0.8cm	Multifocal, stromal bone formation	Papillary carcinoma	I	Wild
45.	915/09	50/M	Euthyroid	2.0cm	Extrathyroidal extension	Papillary carcinoma	III	Wild
46.	920/09	59/M	Euthyroid	1.0-3.0cm	Multifocal	Papillary carcinoma	II	Wild
47.	1218/09	32/F	Euthyroid	1cm	Cystic	Papillary carcinoma	I	Mutant
48.	2548/09	20/F	Euthyroid	3.5cm	Multifocal, capsular and lymph node involvement	Papillary carcinoma	III	Mutant
49.	2662/09	40/F	Euthyroid	11.5cm	Extra thyroidal extension	Anaplastic carcinoma	IVB	Wild
50.	3561/09	45/F	NA	3.5cm	Lymph node involvement	Papillary carcinoma	III	Wild

FN- UMP – Follicular neoplasm of uncertain malignant potential;

NE- DNA Not Extracted.